CLAIMS

1. A polynucleotide encoding a protein that comprises mycobacterium paratuberculosis acylase (*mpa*), or a fragment or homologue of said protein, said fragment or homologue having *mpa* activity.

2. A polynucleotide selected from:

(a) a polynucleotide comprising the entire nucleotide sequence set out in SEQ ID No. 1 or the complement thereof;

(b) a polynucleotide comprising a nucleotide sequence capable of hybridising to a fragment of the nucleotide sequence set out in SEQ ID No. 1, the fragment having the nucleotide sequence of nucleotides 210-1335 of SEQ ID No. 1;

(c) a polynucleotide comprising a nucleotide sequence capable of hybridising to the complement of a fragment of the nucleotide sequence set out in SEQ ID No. 1, the fragment having the nucleotide sequence of nucleotides 210-1335 of SEQ ID No. 1;

(d) a polynucleotide comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotide of SEQ ID No. 1 or a polynucleotide of (c); and

(e) a polynucleotide having at least 80% homology to the nucleotide sequence of SEQ ID No. 1.

3. A polynucleotide according to claim 2 which encodes a polypeptide having *mpa* activity.

4. A polynucleotide according to claim 1 or 3 wherein the *mpa* activity is the acetylation of cell wall components.

5. A polynucleotide probe or primer which comprises a fragment of at least 15 nucleotides of a polynucleotide selected from:

(b) a polynucleotide comprising a nucleotide sequence capable of hybridising to a fragment of the nucleotide sequence set out in SEQ ID No. 1, the fragment having

the nucleotide sequence of nucleotides 210-1335 of SEQ ID No. 1;

(c) a polynucleotide comprising a nucleotide sequence capable of hybridising to the complement of a fragment of the nucleotide sequence set out in SEQ ID No. 1, the fragment having the nucleotide sequence of nucleotides 210-1335 of SEQ ID No. 1; and

(d') a polynucleotide comprising a polynucleotide sequence which is degenerate as a result of the genetic code to a polynucleotide sequence of (c).

6. A polypeptide in substantially isolated form which is encoded by a polynucleotide of any one of claims 1 to 4.

7. A polypeptide in substantially isolated form which comprises the sequence set out in SEQ ID No. 2, or a polypeptide substantially homologous thereto which has *mpa* activity, or a fragment of the polypeptide of SEQ ID No. 2 which has *mpa* activity.

8. A polypeptide according to claim 7 which has the sequence set out in SEQ ID No. 2.

9. A polypeptide comprising at least 8 amino acids which is an immunogenic fragment of a polypeptide defined in claim 7 or 8 and which comprises an epitope which is specific to the pathogenicity of mycobacterial cells.

10. A vector comprising a polynucleotide as defined in any one of claims 1 to 4.

11. An expression vector comprising a polynucleotide as defined in any one of claims 1 to 4, operably linked to regulatory sequences capable of directing expression of said polynucleotide in a host cell.

12. An antibody capable of recognising a polypeptide as defined in any one of claims 6 to 9.

13. An antibody according to claim 12 which is a monoclonal antibody or a fragment thereof.

14. A method for detecting the presence or absence of a polynucleotide as defined in any one of claims 1 to 4 in a biological sample which method comprises:

(a) bringing a biological sample containing DNA or RNA into contact with a probe according to claim 5 under hybridising conditions; and

(b) detecting any duplex formed between the probe and nucleic acid in the sample.

15. A method of detecting the presence or absence of a polypeptide as defined in any one of claims 6 to 9 in a biological sample which method comprises:

(a) incubating the biological sample with an antibody according to claim 12 or 13 under conditions which allow for the formation of an antibody-antigen complex; and

(b) determining whether antibody-antigen complex comprising said antibody is formed.

16. A method of detecting the presence or absence of antibodies in a biological sample which method comprises:

(a) incubating a biological sample with a polypeptide according to any one of claims 6 to 9 comprising an epitope under conditions which allow for the formation of an antibody-antigen complex; and

(b) determining whether an antibody-antigen complex comprising said polypeptide is formed.

17. A method of detecting the presence or absence of cell mediated immune reactivity in an animal or human, to a polypeptide according to any one of claims 6 to 9 which method comprises:

(a) incubating a cell sample with a polypeptide according to any one of claims 6 to 9 comprising an epitope under conditions which allow for a cellular immune response; and

(b) detecting the presence of said cellular immune response in the incubate.

18. A test kit for detecting the presence or absence of a pathogenic mycobacterium in a sample which comprises a polynucleotide according to any one of claims 1 to 4 or a polypeptide according to any one of claims 6 to 9 or an antibody according to claim 12 or 13.

19. A pharmaceutical composition comprising (i) a polypeptide according to any one of claims 6 to 9 or a polynucleotide according to any one of claims 1 to 4 or an antibody according to claim 12 or 13 and (ii) a suitable carrier or diluent.

20. A polypeptide according to any one of claims 6 to 9 or a polynucleotide according to any one of claims 1 to 4 or an antibody according to claim 12 or 13, for use in the treatment, prevention or diagnosis of a disease caused by a mycobacterium.

21. A method of treating or preventing a mycobacterial disease in an animal or human caused by mycobacteria which express a polypeptide according to any one of claims 6 to 9, which method comprises administering to the animal or human an effective amount of said polypeptide.

22. A method of treating or preventing a mycobacterial disease in animals or humans caused by mycobacteria containing the nucleotide sequence of SEQ ID No. 1, which method comprises administering to the animal or human an effective amount of a polynucleotide according to any one of claims 1 to 4 or a vector according to claim 10 or 11.

23. A method according to claims 21 or 22 wherein the mycobacterial disease is Johne's disease or Crohn's disease.

24. A method according to any one of claims 21 to 23 for increasing the in vivo susceptibility of mycobacteria to antimicrobial drugs.

25. A vaccine composition comprising (i) a polypeptide according to any one of claims 6 to 9 or a polynucleotide according to any one of claims 1 to 4 or a vector according to claims 10 or 11 together with (ii) a pharmaceutically acceptable carrier or diluent.

26. A plasmid containing a polynucleotide sequence according to any one of claims 1 to 4 under the control of a promoter.

27. A plasmid according to claim 26 wherein the promoter is a CMV, MMLV, RSV or SV40 promoter.

28. A nucleic acid vaccine comprising (i) a plasmid as defined in claim 26 or 27 and (ii) a pharmaceutically acceptable carrier or diluent.

29. A vaccine according to claim 28 which further comprises a transfection agent.

30. A vaccine comprising (i) a polypeptide as defined in any one of claims 6 to 9, optionally linked to a hapten molecule, and (ii) a pharmaceutically acceptable carrier or diluent.

31. A non-pathogenic microorganism or a cell from a human or animal species prone to infection by *mpa*-containing mycobacteria comprising a component on its surface which has been modified by a polypeptide according to any one of claims 6 to 9.

32. A non-pathogenic microorganism or a cell from a human or animal species prone to infection by *mpa*-containing mycobacteria which has been transformed or transfected with a nucleic acid construct comprising a polynucleotide as defined in any one of claims 1 to 4 and 26 to 29.

33. A non-pathogenic microorganism or a cell from a human or animal species prone to infection by *mpa*-containing mycobacteria wherein the nucleic acid construct according to

claim 32, further comprises a polynucleotide which encodes the polypeptides of the GS region of *MAP*.

34. A non-pathogenic microorganism or a cell from a human or animal species prone to infection by *mpa*-containing mycobacteria according to claim 32 or 33 wherein the gene or genes present in the nucleic acid construct are expressed.

35. A vaccine comprising (i) a non-pathogenic microorganism or a cell from a human or animal species prone to infection by *mpa*-containing mycobacteria according to any one of claims 31 to 34 and (ii) a pharmaceutically acceptable carrier or diluent.

36. A non-pathogenic microorganism or a cell from a human or animal species prone to infection by *mpa*-containing mycobacteria comprising on its surface an antigenic determinant capable of being produced by the action of a polypeptide as defined in claim 7 or 8 and which is capable of eliciting antibodies which bind the surface of *MAP*.

37. A normally pathogenic mycobacterium or pathogenic isolate thereof, whose pathogenicity is mediated in all or in part by the presence or expression of a polypeptide as defined in any one of claims 6 to 9, which mycobacterium or isolate harbours an attenuating mutation in the polynucleotide sequence as defined in any one of claims 1 to 4.

38. A vaccine comprising (i) a non-pathogenic microorganism or cell from a human or animal species as defined in claim 36 or a mycobacterium or isolate as defined in claim 37, and (ii) a pharmaceutically acceptable carrier or diluent.

39. A vaccine according to claim 38 which comprises a mycobacterium or isolate wherein the attenuating mutation in the mycobacterium or isolate is mediated by the insertion of one or more nucleotides.

40. A polynucleotide insertion element selected from:

(a) a polynucleotide comprising the nucleotide sequence set out in SEQ ID Nos. 3 or 4;

(b) a polynucleotide comprising a nucleotide sequence capable of hybridising to a fragment of the nucleotide sequence set out in SEQ ID No. 3, the fragment having the nucleotide sequence of nucleotides 1856-2543 of SEQ ID No. 3;

(c) a polynucleotide comprising a nucleotide sequence capable of hybridising to a fragment of the nucleotide sequence set out in SEQ ID No. 4, the fragment having the nucleotide sequence of nucleotides 1-688 of SEQ ID No. 4;

(d) a polynucleotide comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotide of SEQ ID No. 4 or a polynucleotide of (b);

(e) a polynucleotide having at least 75% homology to the nucleotide sequence of SEQ ID No. 3; and

(f) a polynucleotide having at least 75% homology to the nucleotide sequence of SEQ ID No. 4.

41. A vaccine according to claim 39 wherein the sequence of one or more nucleotides is a sequence as defined in claim 40.

42. A polynucleotide probe or primer which comprises a fragment of at least 15 nucleotides of a polynucleotide selected from:

(b) a polynucleotide comprising a nucleotide sequence capable of hybridising to a fragment of the nucleotide sequence set out in SEQ ID No. 3, the fragment having the nucleotide sequence of nucleotides 1856-2543 of SEQ ID No. 3;

(c) a polynucleotide comprising a nucleotide sequence capable of hybridising to a fragment of the nucleotide sequence set out in SEQ ID No. 4, the fragment having the nucleotide sequence of nucleotides 1-688 of SEQ ID No. 4; and

(d') a polynucleotide comprising a polynucleotide sequence which is degenerate as a result of the genetic code to a polynucleotide of (b).

43. A polypeptide in substantially isolated form which is encoded by a polynucleotide of claim 40.

44. A polypeptide comprising at least 8 amino acids which is an immunogenic fragment of the polypeptide defined in claim 43 and which comprises an istA epitope.

45. A vector comprising a polynucleotide as defined in claim 40.

46. An expression vector comprising a polynucleotide as defined in claim 40, operably liked to regulatory sequences capable of directing expression of said polynucleotide in a host cell.

47. A method for preparing a mycobacterium or pathogenic isolate as defined in claim 37 which method comprises transfecting animal or human isolate of an *mpa* containing pathogenic bacterium with a polynucleotide construct comprising a polynucleotide as defined in claim 40.

48. The method of claim 47 wherein transfection is effected by electroporation.

49. The method of claim 47 or 48 wherein the polynucleotide sequence as defined in claim 40 has the nucleotide sequence set out in SEQ ID No. 3 or 4.